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STUDIES OF METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF--ETC(U)
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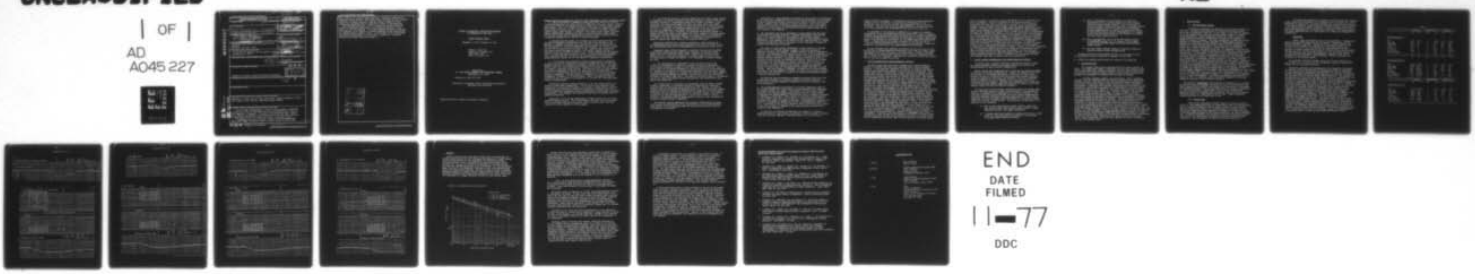

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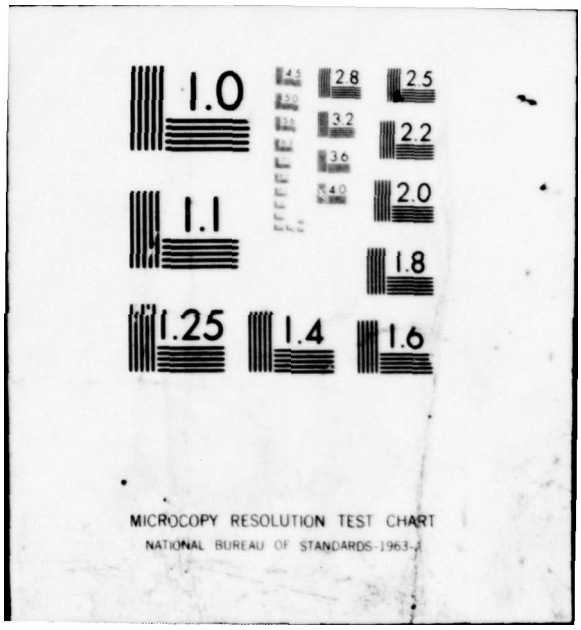
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We have shown that propranolol reduces oxygen binding by hemoglobin in intact red cells by increasing the selective permeability of the red cell membrane to potassium. The exodus of potassium and water results in an increase in hydrogen ion concentration in the cell and a reduction in hemoglobin affinity for oxygen as a result of the Bohr effect.

In patients with acute myocardial infarction, we have found that whole body oxygen consumption remains constant despite a nearly three-

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fold variation in arterial oxygen flow rate. Although a reduction in hemoglobin oxygen affinity could explain about one third of the increased extraction of oxygen required to maintain oxygen consumption other compensating mechanisms must be important for most of the adaptation.

We have found that water soluble radiographic contrast materials produce a significant alteration in the Donnan distribution of ions across the red cell membrane. The normal negative potential of the red cell membrane can be reduced or reversed depending on the concentration of contrast material added to blood. As the inside of the cell becomes more positive with respect to the outside, proteins are, in effect, repelled into plasma producing acidemia.

STUDIES OF METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF RED CELLS

ANNUAL PROGRESS REPORT

September 1, 1974 to October 31, 1975

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1. The relationships of hemoglobin concentration and blood pH to red cell 2,3-diphosphoglycerate and oxygen binding by hemoglobin have been studied in healthy subjects and subjects with hypoproliferative anemia with or without severe chronic renal disease. Red cell 2,3-DPG was inversely correlated with hemoglobin deficit and directly and equally strongly associated with blood pH in anemic subjects without chronic renal disease. In subjects with chronic renal disease receiving regular hemodialysis, predialysis pH was not increased despite severe anemia, and red cell 2,3-DPG was not significantly elevated, except in subjects who had a sustained alkalosis due to the use of sodium bicarbonate.

In hypoproliferative anemia, the increment in pH was associated with the decrease in hemoglobin concentration such that 80% of the increase in P_{50} measured at standard conditions which occurred with anemia was explicable by the relationship of (a) pH with hemoglobin concentration, (b) red cell 2,3-DPG with pH, and (c) P_{50} std with red cell 2,3-DPG. However, P_{50} at the pH and base excess present *in vivo* was similar in all anemic subjects whether an increase in red cell 2,3-DPG occurred or not. Blood alkalosis and the accumulation of 2,3-DPG cancelled each other's effect on oxygen binding by hemoglobin. Hence, increased red cell 2,3-DPG and P_{50} compensated for the alkalosis of hypoproliferative anemia, not for the deficit in hemoglobin concentration.

2. We have shown that propranolol reduces oxygen binding by hemoglobin in intact red cells by increasing the selective permeability of the red cell membrane resulting in an exodus of potassium, chloride, and water. The latter effects result in a new distribution of hydrogen ion between cell and plasma, and thereby a reduction in red cell pH. The reduction in pH can fully explain the change in hemoglobin's affinity for oxygen based on the Bohr effect. Either D- or DL-propranolol can produce the change in red cell pH and oxygen binding by hemoglobin. The drug action on permeability is not prevented by epinephrine, although it is by chlorbutanol. Hence, the membrane action of propranolol does not appear to be related to its activity as a beta-adrenergic receptor blocking agent.

Propranolol produced a marked alteration in red cell shape as well as in hydration (hypovolumic stomatocytes). The two effects were separable since dehydration of the cell by the addition of sucrose to plasma did not result in stomatocytes and chlorbutanol blocked the enhancement of permeability of the red cell membrane by propranolol without preventing the shape change (isovolumic stomatocytes). This suggests that propranolol may have two separate sites of membrane interaction.

Propranolol (10 to 360 mg) administered to human subjects did not affect hemoglobin-oxygen affinity. This is explained by the fact that the concentration in blood after such doses is nearly 4,000 to 100-fold lower than that required to achieve changes in blood *in vitro*.

3. The interrelationships of arterial oxygen flow rate index, oxygen binding by hemoglobin, and oxygen consumption have been examined in patients with acute myocardial infarction. Proportional extraction of oxygen increased in close association with decreasing oxygen flow rate, and hence, whole body oxygen consumption was constant over nearly a three-fold variation in arterial oxygen flow rate. A reduction in hemoglobin-oxygen affinity at in vivo conditions of pH, Pco_2 and temperature also occurred in proportion to the reduction in arterial oxygen flow rate. Therefore, the increased proportional removal of oxygen from arterial blood at low oxygen flow rates, required to maintain oxygen consumption, may have been facilitated by the reduced affinity of hemoglobin for oxygen at in vivo conditions. However, the decrease in affinity did not appear to explain more than 30-40% of the increased extraction.

Respiratory alkalosis was a frequent occurrence in these patients and 2,3-diphosphoglycerate was positively associated with blood pH as well as with the time-averaged proportion of deoxyhemoglobin in arterial and venous blood.

Hemoglobin-oxygen affinity measured at standard conditions and the mixed venous oxygen saturation were equally good indicators of reduced arterial oxygen flow rate in patients without shock. However, $S\bar{v}o_2$ is more easily measured and is a more useful indicator of reduced oxygen flow rate, since its relationship to oxygen flow appears to be independent of affinity changes and time.

4. The inconvenience of measurement of red cell pH, has led to the practice of establishing the relation of oxygen saturation of hemoglobin to the partial pressure of oxygen in blood based on extracellular pH. This method relies on the precise dependence of intraerythrocytic pH on extracellular pH. Studies of the effects of certain plasma additives on the binding of oxygen to hemoglobin have neglected to consider the possibility that the normal negative potential difference between the interior and exterior of the red cell may be disturbed by the agent under study. The change in potential leads to a concomitant loss in the usual relationship between extracellular and intracellular ionic species and thereby pH.

In a recent study of the effects of radiographic contrast media on oxygen binding to hemoglobin, an increased affinity of hemoglobin for oxygen was observed based on corrections using extracellular pH. However, it would be expected that poorly penetrating compounds would reduce the pH gradient between plasma and cells, by causing an acidification of plasma due to a net movement of hydroxyl ion into the red cell without a concomitant change in red cell pH because of the high buffering capacity of the red cell interior. Hence, a portion of the apparent affinity change would be spurious, since intracellular pH governs oxygen binding to hemoglobin.

The following studies demonstrate how erroneous inferences may be drawn from reliance on extracellular pH for determination of the oxygen-hemoglobin dissociation curve in situations where the ion distribution across the red cell membrane is disturbed.

Construction of oxygen-hemoglobin dissociation curves based on extracellular pH and using blood tonometered with 5% CO_2 was shown misleading in the presence of poorly penetrating non-ionic molecules like sucrose or poorly penetrating anionic compounds like radiographic contrast materials. False conclusions regarding the position of the oxygen-hemoglobin dissociation curve result because of the disturbance of the normal pH gradient between plasma and red cell induced by such chemicals.

5. The development and use of radiographic contrast media have been major contributions to clinical medicine. Although considerable study has been given to the adverse effects of these agents, some reactions remain unexplained. Most research on the adverse effects of water-soluble contrast materials has been directed to the effects of the rapid injection of a hyperviscous and hypertonic bolus on rheology of blood, although a "tendency to acidosis" has been noted previously.

The effect of water-soluble radiographic contrast material on pH when added to blood in clinical dosages in vitro or when used in vivo for diagnostic purposes, was examined. Contrast material caused a reduction of blood pH. The mechanism of this occurrence was found to be the balancing of the negative charge of intracellular organic anions by the extracellular anionic contrast material molecules. The normal negative potential of about 10 millivolts across the red cell membrane was reduced, nullified or reversed depending on the concentration of contrast material, added to blood. As the inside of the cell became more positive with respect to the outside, protons were, in effect, repelled into plasma, although the apparent exodus of protons occurs by the generation and outward diffusion of carbon dioxide. Since the acidemia is dependent on rehydration of carbon dioxide in plasma, a reaction measured in seconds, the site of injection and transit time of dye will contribute to the pH of the plasma during passage through a regional capillary bed.

We speculate that an alteration in membrane potential and/or the acute acidemia may contribute to the adverse effects of contrast material, particularly on tissues dependent on membrane electrical rhythmicity such as the myocardium.

6. The oxygen-hemoglobin equilibrium is of clinical importance since it may provide evidence for a structurally altered hemoglobin with heightened or lessened affinity for oxygen. The presence of a structurally altered hemoglobin with abnormal oxygen binding characteristics is made evident by examining the oxygen-hemoglobin dissociation curve at standard conditions of temperature, pH and Pco_2. The position of the curve is represented by the P_{50} standard (P_{50} std), that is the Po_2 at which hemoglobin is half-saturated with oxygen at standard conditions. Standardization eliminates the effects of variations in temperature, pH and the pH-independent effect of Pco_2. Deviations in the P_{50} std, therefore, imply either an altered content of red cell 2,3-DPG or a hemoglobin with oxygen binding characteristics different from hemoglobin A.

In addition, the affinity of hemoglobin for oxygen is of clinical importance because it may decrease in response to hypoxia, anemia or reduced blood flow and acts, thereby, to maintain venous (i.e. tissue) Po_2 as

oxygen extraction increases. In situations in which the oxygen-hemoglobin content or flow, the effects of the four major determinants of the equilibrium, that is red cell 2,3-DPG, pH, temperature, and Pco_2 must be considered. This has been called the oxygen-hemoglobin dissociation curve at in vivo conditions, and is presented by the P_{50} in vivo (P_{50} i.v.).

An assessment of the affinity of hemoglobin for oxygen is considered inaccessible to the practicing physician and hematologist since it requires tonometry and mixing techniques often available only in research laboratories. In the following studies, we examined the usefulness of a single venous blood sample as an indicator of the position of the oxygen-hemoglobin dissociation curve. A single venous blood sample, analyzed for pH, Po_2, and So_2 by a clinical chemistry laboratory could be used by any physician to assess the presence of an alteration in the oxygen-hemoglobin equilibrium.

We have validated this technique correlating the P_{50} std or P_{50} in vivo derived from an oxyben-hemoglobin dissociation curve with that from a single measurement of pH, Po_2, Pco_2 and So_2 in venous blood. Studies of subjects with alkalosis or acidosis and with high and low affinity hemoglobins were made to verify this technique. Equations were developed to allow the calculation of P_{50} from a single Po_2 and So_2.

7. Atrial tachypacing and oxygen-hemoglobin affinity.

The first study deals with atrial tachypacing and oxygen-hemoglobin affinity in patients with chest pain syndromes, most often with angina pectoris. It was conceived originally in response to research reported by Shappell and coworkers and referred to earlier in this application. Their studies showed that 6 patients with coronary disease who underwent atrial tachypacing, only the 5 who developed chest pain showed an increase during pacing in P_{50} standard from arterial to coronary sinus blood. The increase was independent of DPG, ATP, or red cell pH—the known determinants of P_{50} standard—and suggested the possibility that a substance was released during ischemia which produced the decrease in oxygen hemoglobin affinity across the coronary bed. Because of the potential significance of this finding (in particular if right shifted curves are in fact advantageous in ischemic states), and because investigations in our laboraotry indicated that P_{50} standard correlated very strongly with DPG alone, we were interested in further exploring this question in patients undergoing atrial tachypacing studies for diagnostic purposes in our cardiac catheterization laboratory, where routine coronary sinus pacing makes the requisite blood sampling possible and convenient. Thus far, 11 patients, 9 with coronary artery disease and pacing induced angina, and 2 with chest pain and normal coronary arteries, have been studied in control, paced, and recovery states. An initially surprising (and to our knowledge new) observation, is that virtually each patient developes alkalosis during pacing, and this pH change is on a respiratory basis (pCO_2 falls appropriately). A rise in arterial pO_2 from the rest to the paced state occurred in many patients and is consistent with this increase in respiration. As a consequence of the pH change, P_{50} at <u>in vivo</u> pH (calculated from P_{50} standard) is <u>decreased</u> during atrial tachypacing. Unlike the prior investigations, we have not detected during the paced state any increase in P_{50} standard from artery to coronary sinus. Without enumerating the several ways in which apparently differing results

can be reconciled, the results of this study appear to be of interest in at least two respects. First, as indicated in the original proposal, it is conceivable that when flow is fixed (as it well may be in the regionally involved areas in coronary heart disease), and when oxygen extraction is maximum, a leftward shift in the in vivo oxygen hemoglobin dissociation curve (such as that induced by alkalosis) may be detrimental to oxygen transport. Making several assumptions (regional flow is fixed, venous pO_2 is proportionate to tissue pO_2, and for both there is a critical ischemic level, and capillary pO_2 is rate limiting for oxygen transport to ischemic tissue), we calculated that the patient with the largest decrease in P_{50} in vivo (arterial, coronary sinus, and averaged) during pacing might have decrease his oxygen extraction and therefore his oxygen transport by 10% as a result of the alkalosis. Using this reasoning, it is conceivable that pH through its effect on oxygen delivery (just as rate pressure product has its effect on oxygen demand) is important in determining threshold for angina. Second, it is also conceivable that in spontaneous angina there may be pH (alkalosis) antecedents, just as there are blood pressure and heart rate antecedents, and that these may effect threshold for pain by impairing oxygen transport. Pilot studies are planned monitoring depth and rate of respiration in hospitalized patients with ischemic chest pain to explore this latter possibility.

8. Chronic cardiac decompensation and oxygen-hemoglobin affinity

A clinical study is underway exploring relationships between 2,3-DPG, pH, oxygen hemoglobin affinity, and oxygen transport in patients with varying degrees of chronic cardiac decompensation.

Earlier studies have established that 2,3-DPG and P_{50} standard increase in patients with cardiac decompensation. Woodson and coworkers noted that the increase in P_{50} standard was greater in patients with more severe disease, and that it correlated best with mixed venous oxygen saturation. pH was not measured in this study, and therefore, its role in producing affinity changes could not be assessed, and P_{50} in vivo could not be calculated from P_{50} standard. Rosenthal and coworkers noted a significant inverse relationship between oxygen flow index and 2,3-DPG, but as in the previous study, the role of pH was not assessed.

The study which we have planned, involving patients with chronic heart disease undergoing diagnostic cardiac catheterization at Strong Memorial Hospital, is designed to be comprehensive in a fashion analogous to that previously reported involving patients with acute cardiac dedompensation following myocardial infarction (including measurement of cardiac index, hemoglobin, arterial and mixed venous blood gases, and arterial and mixed venous red cell ATP, 2,3-DPG and pH). In particular, the questions to be explored are the following:

(1) What are the interrelations between arterial oxygen flow rate (cardial index times arterial oxygen content), oxygen hemoglobin affinity, and oxygen consumption?

(2) If oxygen extraction increases as cardiac index falls, to what extent can this extraction be accounted for in decreases in in vivo oxygen hemoglobin affinity?

(3) What is responsible for the increase in 2,3-DPG which accompanies a fall in arterial oxygen flow index (cardiac index and arterial oxygen content)? Does the increase in 2,3-DPG correlate with blood pH (arterial or averaged arterial and mixed venous)?, does it correlate with time averaged percent deoxyhemoglobin in arterial and mixed venous blood? By implication,is pH or percent deoxyhemoglobin or a combination of both the driving force for the increase in 2,3-DPG? Are there other factors involved.

(4) What is in vivo P50? How do pH and DPG changes interact in determining in vivo P_{50}? Do changes in P_{50} in vivo correlate with arterial oxygen flow index and/or arterial oxygen content?

(5) How does oxygen transport compare in equivalent degrees of acute and chronic cardiac decompensation?

Fifteen patients have been studied thus far, but the results are in too preliminary a state of analysis for comment at this time.

9. Cardiac and skeletal muscle models for studies of O_2 metabolism

a. Myocardial model

The coronary sinus could be cannulated and the cannula sutured in place. In a significant number of instances, the left main coronary artery could be dissected, and a catheter introduced from the carotid artery could be sutured in the dissected coronary vessel. Atrial pacing, epicardial ST mapping, and measurement of pressures were performed as planned.

Two major problems, however, were encountered. First, catheters available from our animal research and cardiac catheterization laboratories offered too great a resistance to flow and prevented adequate perfusion of the coronary circulation; cardiac arrest followed soon after cannulation. Subsequently, a stainless steel modified Gregg cannula has been made to order according to specifications provided by Dr. Peter Maroko, but this cannula has not been used as yet. Second, no satisfactory way was found to produce an in vivo change in either DPG or P_{50}. Inosine, phosphate, and pyruvate, in .1 Molar concentrations each produced no change in DPG in the dog, presumably because of the absence in this species of the enzyme erythrocyte purine neucleoside phosphorylase (required for the transformation of inosine into ribose phosphate and then into 2,3-DPG). Sodium bicarbonate in daily intravenous doses, did increase 2,3-DPG levels, but correction of the alkalosis to a stable pH in the normal range with ammonium chloride prior to the acute experiment proved difficult. pH alone could not be used to induce an in vivo affinity change because of the varied effects of pH itself. For these two reasons--initially, lack of a suitable perfusion cannula and later, the absence of a suitable means of inducing in vivo cianges in P_{50} and, in addition, because practical considerations suggested working with one model at a time, the major portion of our effort in the laboratory has been devoted to developing the gracilis muscle model, described below, where progress has been more rapid. When the gracilis studies are well underway, we plan to continue to work with the coronary model.

b. Gracilis model

(i) The experimental design

The design is essentially as described in the contract proposal of 1974-1975. Dissection and cannulation are now performed with facility. Blood flow is arbitrarily fixed at 5 cc per minute, a flow which is adequate for tissue oxygenation at rest and at which oxygen transport is limited during contraction. Sodium bicarbonate rather than saline is used for volume expansion during the exsanguination because of the tendency for metabolic acidosis to develop. Red cells resuspended in plasma have proven superior as a perfusate to those suspended in Krabs Ringers solution. Oxygenation of the blood and pCO_2 control are achieved by bubbling a gas mixture of 4% carbon dioxide and 96% air through two Dow oxygenators in series (Dow - Beaker Gas Permeator - D/HFG-1 silicone hollow fibers). (Pilot studies in our laboratory showed that one oxygenator did not profice adequate gas exchange to maintain equilibrium with perfusate flowing through the system at 5 cc per minute. With two in series, equilibrium could be reached within 30-45 minutes and maintained after flow was initiated). To further improve pH control, (beyond the substantial control achieved with pCO_2 regulation), a Radiometer titrator (titrator - type TTT1C and autoburette type ABU-1B), is now being incorporated into the system. Perfursate is warmed to 32° by passing perfusion lines through a constant temperature bath and this temperature matches closely that of the muscle, which is maintained by radiant heat and measured with needle probes in the muscle. (Alterations in design are planned to bring the temperature of the experiment to 37°). Resting oxygen consumption of the muscle, perfused and maintained in this fashion is 1.2 µl/min-gm (Mean, S.E. .15, n=11). This value obtained at 32° (if a Q10 of 2 is taken as a reasonable estimate), is comparable to that described at 37° by other investigators. The muscle autoregulates appropriately to changes in flow, to occlusion, and to contraction (examples of this autoregulation are shown in the "controls" section).

With regard to contraction, the 20 minutes of stimulation at 2 twitches per second, which was originally proposed, has proven to be excessive (the muscle cannot maintain tension, and recovery from contraction is prolonged). The experimental protocol has now been modified to 45 seconds of contraction at 1 twitch per second, a format where twitch tension is maintained, metabolic recovery is achieved wihtin 30 minutes, and recovery of vascular resistance occurs in a significantly shorter time. Oxygen consumption during contraction is 5-10 times that at rest.

(ii) Control runs

Control runs using blood untreated except for initial titration to pH 7.4, have demonstrated that in a given muscle, each of three successive sets of 45 seconds contraction and 30 minutes recovery is quite similar. They are similar with regard to (1) autoregulation to occlusion prior to contraction (magnitude of the response to a standard 30 seconds occlusion). (2) resting oxygen consumption and resting metabolism, (3) tension generated, fall in vascular resistance, and increase in the metabolism during contraction, and (4) the time course of recovery of vascular resistance after contraction. The following is the data from one such control run:

The muscle was prepared in the manner just described and the aforementioned protocol was followed for four successive sets of contraction. Autoregulation in response to a 30 second occlusion was tested prior to each set, a five minute period of recovery from the occlusion followed, then 45 minutes of one contraction per second, followed by a 30 minute recovery period, at which time autoregulation to occlusion was tested again and a cycle repeated. The muscle temperature was 32°, flow fixed at 4.8 cc/min, and hemoglobin 12 grams/100 ml.

Metabolism

Table 1 shows the metabolic data for the experiment. "Contraction" samples were obtained during the last 15 seconds of contraction and the first 30 seconds of recovery. The values for the arterial sample during contraction are assumed to be identical to the arterial control (i.e., $A_1 = A_2$, $A_4 = A_5$, $A_7 = A_8$; this has in fact, been documented in pilot studies). Recovery samples from one set of contraction are used as controls for the next (i.e., A_3, V_3 is also taken as A_4, V_4; the latter values, not actually measured, are placed in parentheses). Metabolic data was not obtained for the fourth set in which inadequate volume of perfusate was available for the recovery period.

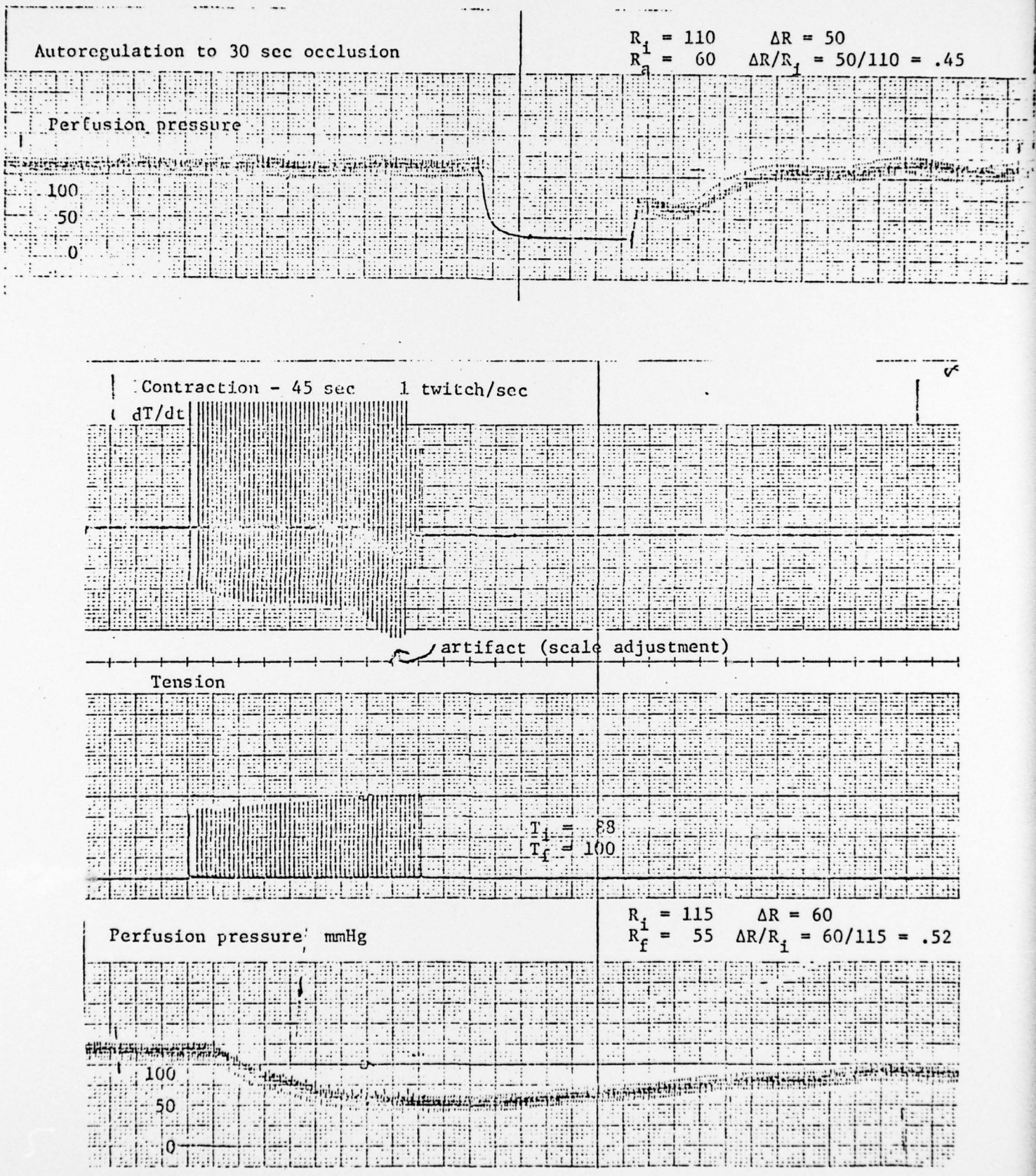
The remarkable similarity between control and recovery samples (i.e., A_1, V_1 and A_3, V_3, A_4, V_4 and A_6, V_6) and between successive "contraction" samples (V_2, V_5, V_8) should be noted.

Mechanical performance and autoregulation--The subsequent four pages--one page for each of the four sets of contraction--consist of photocopies of the original Brush recordings (Clevite-Brush--Mark 260) of perfusion pressure and muscle tension during autoregulation to occlusion, contraction, and the early phase of recovery (paper speed 1mm/second). The response to autoregulation (at the top of the page) occurs prior to contraction (at the bottom), and the tracings are not continuous. This autoregulation to occlusion is quanitified in terms of initial perfusion pressure (proportional to initial resistance, since flow is constant, and labelled R_i), minimal perfusion pressure upon reinstitution of flow (R_a), $\Delta R = R_i - R_a$, and $\Delta R/R_i$. The scale for recording tension is determined at the beginning of the experiment; at present we are not calibrating tension against a known standard, and while successive sets of contractions can be compared quantitatively with one another, no absolute values can be assigned to the magnitude of the tension generated. The first derivative of tension generated with regard to time, dT/dt, is also obtained, through an electronic differentiating circuit. (The sudden change in dT/dt in the first set of contractions is due to adjustment of the scale while muscle is being stimulated). Tension generated during contraction is expressed as T_i (tension generated in the second twitch) and T_f (tension of the final twitch). The resistance change with the autoregulation to contraction is expressed as R_i (initial perfusion pressure), R_f (perfusion pressure at the end of contraction), $\Delta R = R_i - R_f$, and $\Delta R/R_i$. Again, the reproducibility in successive sets of occlusions and contractions should be noted.

Table 1

	CONTROL		CONTRACTION		RECOVERY	
	A_1	V_1	A_2	V_2	A_3	V_3
Contraction Set #1						
pH	7.42	7.39	--	7.38	7.41	7.40
pO_2 mmHg	175	52	--	21.5	175	41.5
pCO_2 mmHg	49.5	47	--	52	45	48
% sat.	99	85	--	24	99	83.5
Q ml/min	4.8	--	--	4.8	--	4.8
AV O_2 diff.	14%	--	--	75%	--	15.5%
$\dot{V}_{O_2}$ μl/min/gm	1.8	--	--	9.6	--	2.0
	A_4	V_4	A_5	V_5	A_6	V_6
Contraction Set #2						
pH	(7.41)	(7.40)	--	7.40	7.42	7.41
pO_2 mmHg	(175)	(41.5)	--	21	175	46
pCO_2 mmHg	(45)	(48)	--	51	48	48
% sat.	(99)	(83.5)	--	25	100	84
Q ml/min	(--)	(4.8)	--	4.8	--	--
AV O_2 diff.	(--)	(15.5%)	--	74%	--	16%
$\dot{V}_{O_2}$ μl/min/gm	(--)	(2.0)	--	9.5	--	21
	CONTROL		CONTRACTION		RECOVERY	
	A_7	V_7	A_8	V_8	A_9	V_9
Contraction Set #3						
pH	(7.42)	(7.41)	--	7.42	7.38	7.40
pO_2 mmHg	(175)	(46)	--	22	175	42
pCO_2 mmHg	(48)	(48)	-	48	50	45
% sat.	(100)	(84)	--	26	99	81
Q ml/min	(--)	(--)	--	--	--	--
AV O_2 diff.	(--)	(16%)	--	74%	--	18%
$\dot{V}_{O_2}$ μl/min/gm	(--)	(21)	--	9.5	--	2.3

Contraction Set #1



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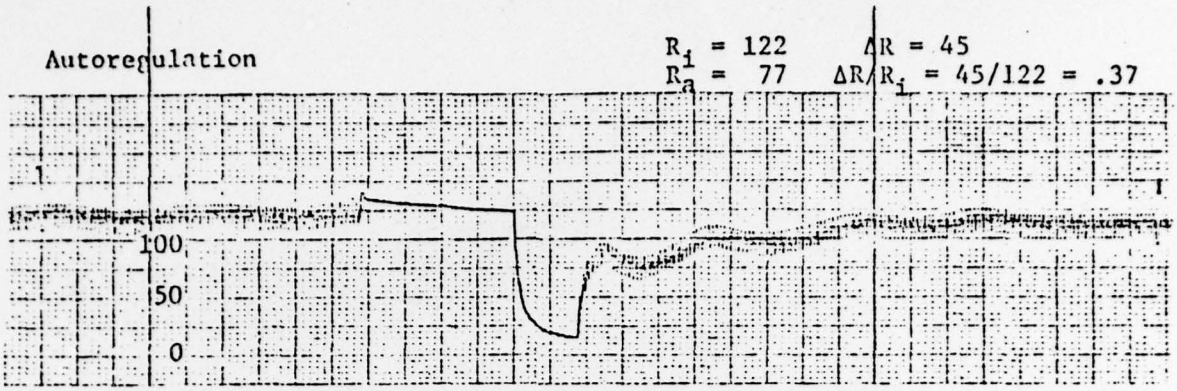

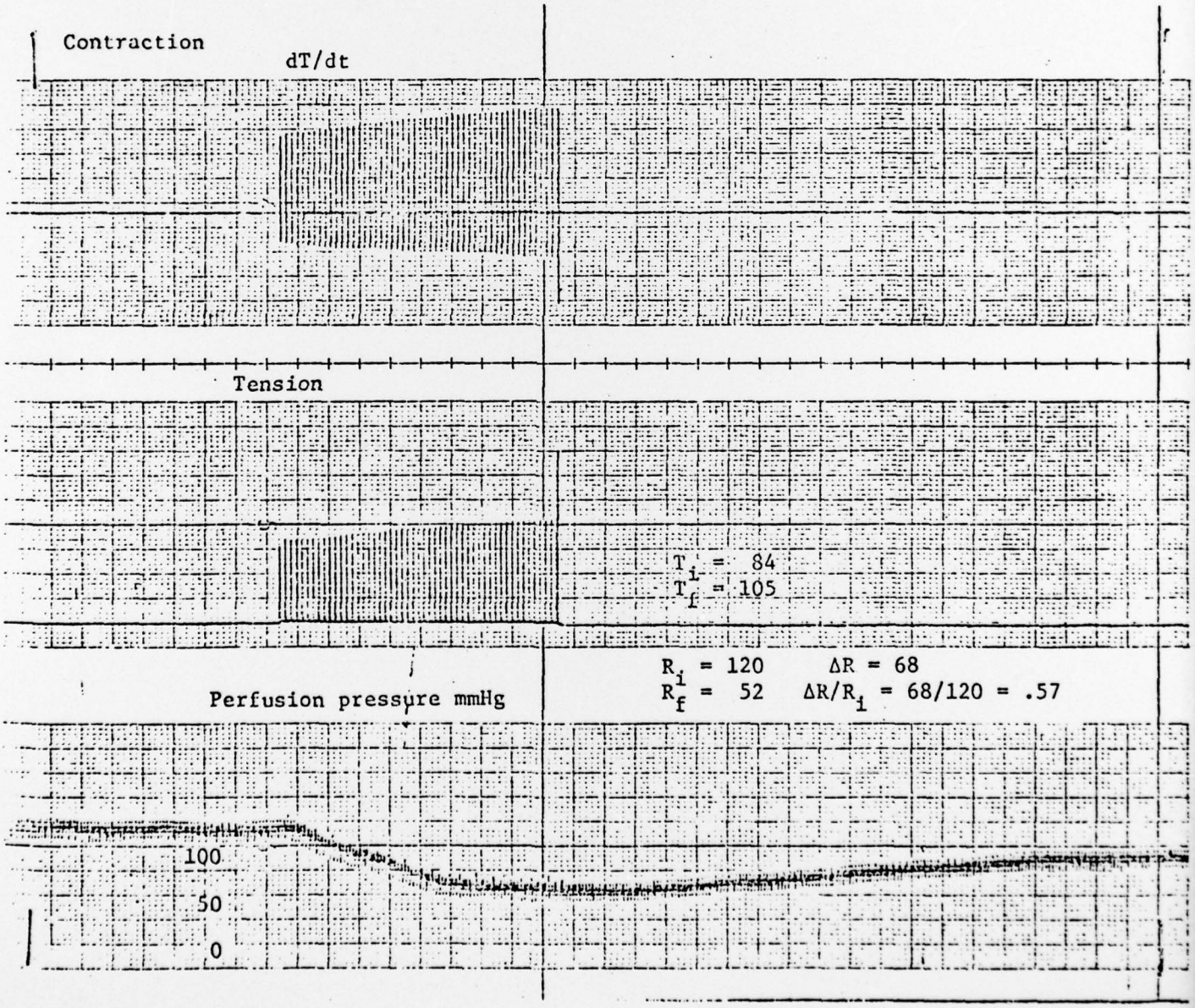

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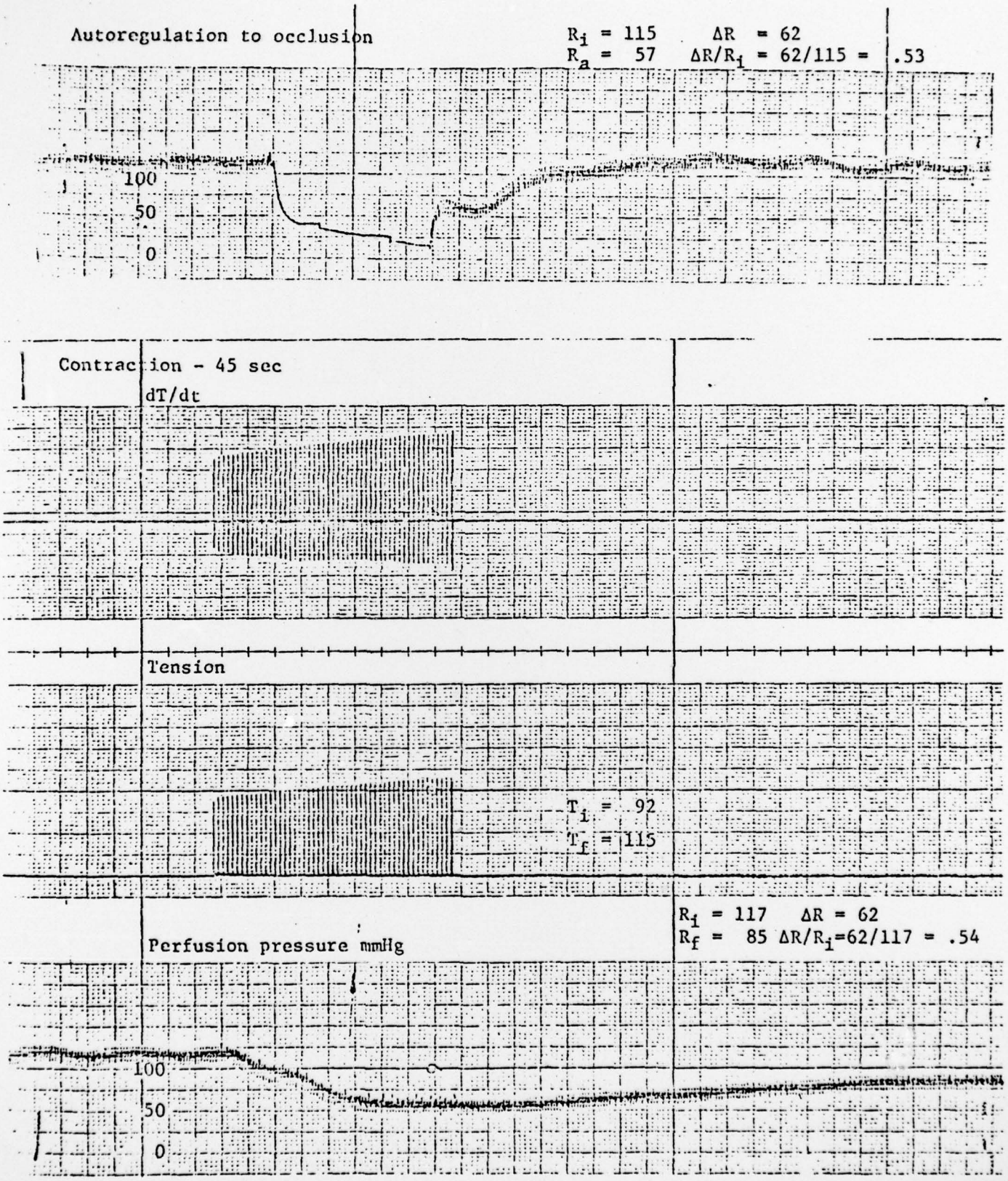
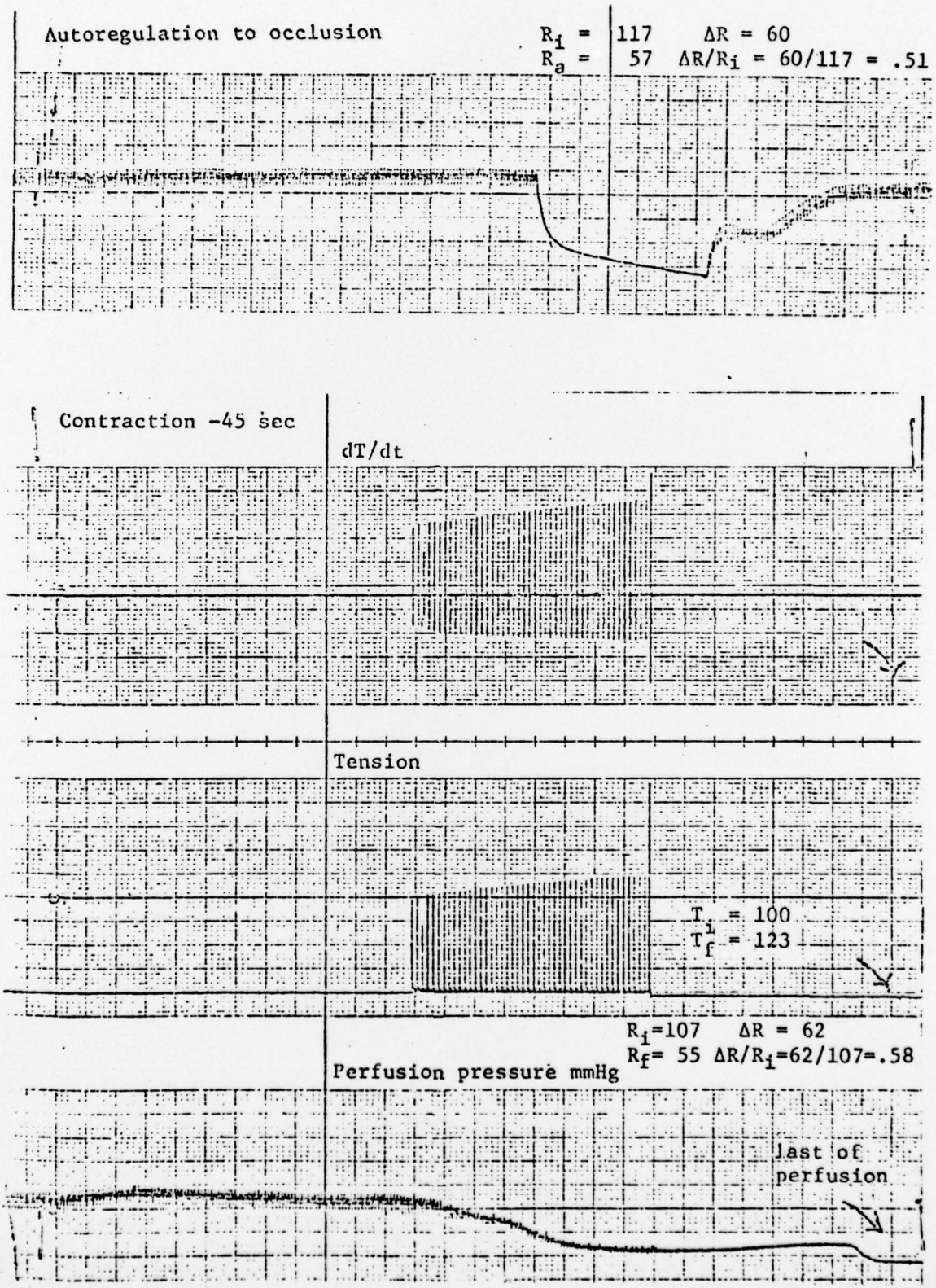
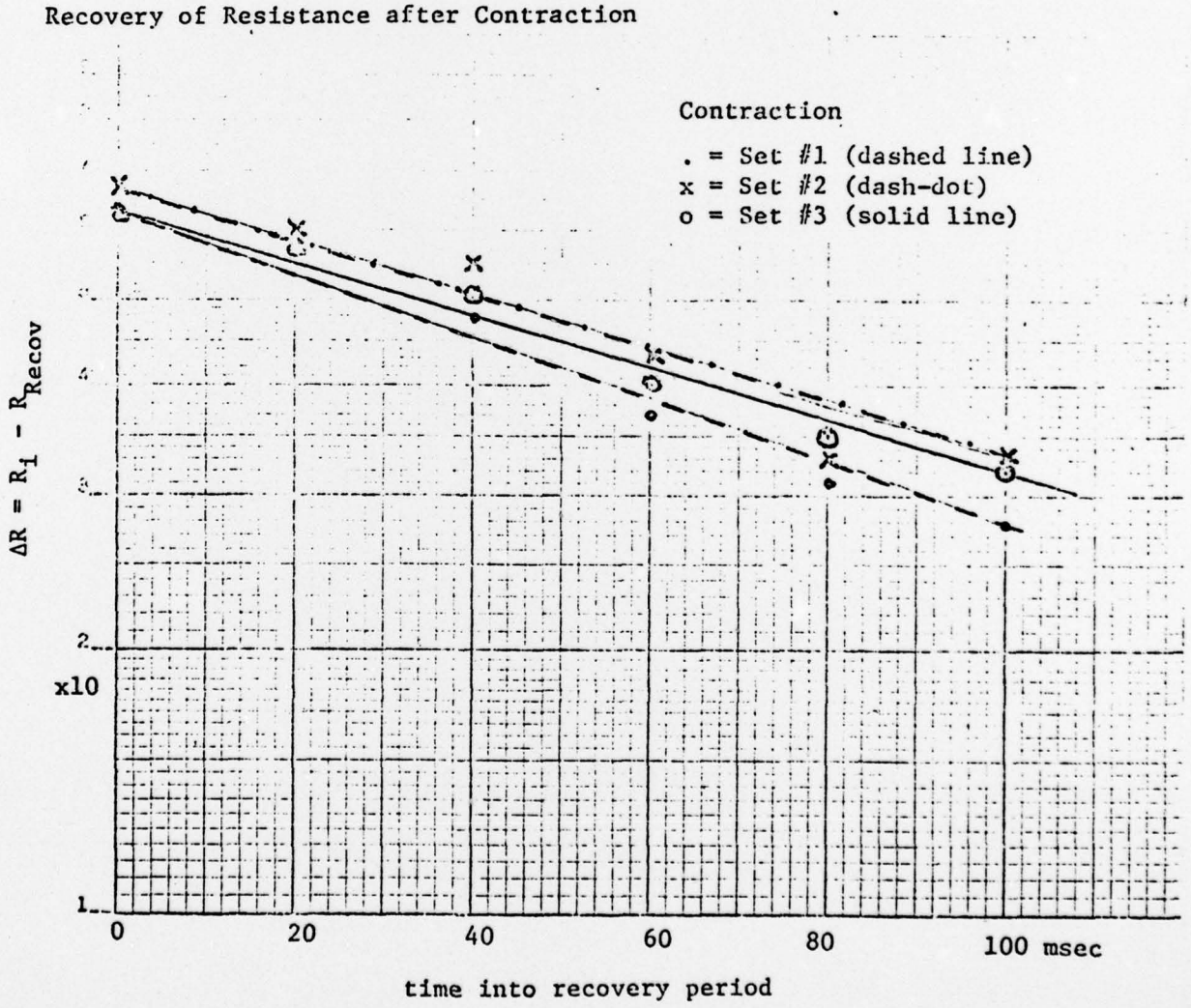


Contraction Set #4



Recovery

Initial recovery phase after each of these sets of contractions is analyzed quantitatively on the following graph (analysis of longer periods of the recovery period are also performed. The difference between perfusion pressure prior to contraction and perfusion pressure during recovery ($R_1 - R_{recov}$) is plotted on the ordinate of the semi-log plot and time into the recovery period is on the abscissa. For this particular graph, the line of the slopes and intercepts of the lines characterizing each of the three recovery phases. One way in which an effect of oxygen-hemoglobin affinity on oxygen transport might manifest itself (should such an effect actually occur) would be an alteration in the slope of the line with represents the "oxygen dependent" portion of this recovery phase.



(Control runs have also been performed in which blood is treated in a manner identical to that employed when potassium cyanate is used to produce an in vivo curve shift, identical except for the absence of the potassium cyanate treatment itself. Blood is centrifuged, the plasma and the white cell layers suctioned off separately, and the cells washed three separate times in a solution containing 130 millimolar sodium chloride, 2.7 millimolar potassium chloride, 10 millimolar glucose and 20 millimolar TES buffer [290 milliosmols, pH 7.4] before being resuspended in plasma with the appropriate adjustment of pH and hematocrit. In control runs using this perfusate, 3 separate sets of contraction have not proven to be as reproducible as those obtained when untreated blood is used. These results are preliminary and are under further investigation. It is conceivable that the centrifugation and decanting which accompanies each of the washings is not as complete as it should be.

(iii) In vitro modification of oxygen-hemoglobin affinity-- Acid citrate-dextrose (ACD) and sodium metabisulfite were used initially, as outlined in the original proposal, but each has its own associated problems. Three other methods in various stages of development now appear more promising.

ACD treated cells are depleted of 2,3-DPG, and oxygen-hemoglobin affinity is consequently increased. We have found that these treated cells, even when in ACD for only one week (ATP levels remain relatively high), and when washed and resuspended in plasma, prove to be a poor perfusate. Perfusion pressure rises rapidly and muscle performance falls off concomitantly suggesting that red cells or cell fragments may be occluding the microcirculation. Phase contrast microscopy of ACD treated cells, both before and after organ perfusion, reveal 1-2+ morphologic changes and 1-2+ rouleaux formation consistent with this hypothesis.

Incubation of red cells with sodium metabisulfite, in our laboratory, produces decreases in 2,3-DPG and P_{50} comparable to those reported by others. In order to get changes of an adequate magnitude (6-8mmHg of P_{50}) requires 3-4 hours of incubation, which is too long a time for the requirements of our experiments.

Potassium cyanate incubation also induces significant decreases in P_{50} std (10mmHg, with no accompanying change in 2,3-DPG) but in contrast to those induced by metabisulfite treatment, these require only two hours. Cyanate treated cells have normal morphology and do not cause elevation of perfusion pressure. Preliminary results indicated that changes in oxygen-hemoglobin affinity induced by potassium cyanate did adversely affect tissue oxygenation during contraction, but adequate controls are only now being obtained, as described in an earlier section. We have been assured by Dr. Anthony Cerami of the Rockefeller University (personal communication) that the exchange of cyanate from treated cells to muscle tissue is insignificant and cannot explain these results.

A contemplated intervention, not included in the original proposal, is the use of carbon monoxyde. Carbon monoxide, in addition to decreasing hemoglobin-oxygen capacity, induces a leftward shift in the oxygen-hemoglobin dissociation curve. By administering carbon monoxyde at 400 parts per million to a donor dog (via an endotracheal tube) and by ventilating with a respirator at 3 liters per minute over 3 hours, we anticipate that blood with 25% carboxyhemoglobin can be obtained, blood with a 7 or 8 mm leftward shift in the oxygen hemoglobin dissociation curve. The cells from a donor dog treated in this fashion can be resuspended in pooled plasma (plasma from donor and recipient--cells from recipient also suspended in pooled plasma) to give a final hematocrit which is 4/3 that of the untreated blood. In this manner, perfusates can be obtained consisting of cells from donor (left shifted by carbon monoxide treatment) and recipient (normal P_{50}) suspended in the same plasma and with the same oxygen capacity, but differing in oxygen hemoglobin affinity. With suitable controls, inherent differences in the cells of the donor and recipient can be accounted for.

A final intervention which is being evaluated is the use of donor sheep blood to perfuse a recipient dog gracilis. There are sheep with naturally differing oxygen hemoglobin affinities (range 13-37mmHg) and the effects of cells from two donors with widely differing affinities suspended in pooled plasma at an appropriate hematocrit could be compared. With the cooperation of the Rochester Zoo, blood from Mufflon sheep has been obtained. the P_{50} standard determined in our laboratory is 24 mmHg virtually identical to that described in the literature (23 mmHg). Dog gracilis muscle performed well, in pilot studies, when the Mufflon blood was used as perfusate. Similar studies using other sheep species are planned. and in vitro studies of the sheep blood are underway to determine red cell pH, intracellular vs. extracellular pH gradient, Bohr coefficient, temperature coefficient for converting P_{50} standard to P_{50} in vivo, and red cell DPG and ATP--information necessary for proper evaluation of this intervention. Similar in vitro studies involving normal dog blood and hemoglobin, and cyanated dog blood and hemoglobin are also underway.

Articles published or in press with support of Contract DA17-73-C-3135 since last progress report:

1. Lichtman, M.A., Murphy, M.S., Whitbeck, A.A. and Kearney, E.A. Oxygen binding to hemoglobin in subjects with hypoproliferative anemia, with and without chronic renal disease: Role of pH. Brit. J. Haematol. 27: 439, 1974.

2. Lichtman, M.A., Cohen, J., Murphy, M.S., Kearney, E.A. and Whitbeck, A.A. Effect of propranolol on oxygen binding to hemoglobin in vitro and in vivo. Circulation 49: 881, 1974.

3. Lichtman, M.A., Cohen, J., Young, J.A., Whitbeck, A.A. and Murphy, M.S. The relationship between arterial oxygen flow rate, oxygen binding by hemoglobin and oxygen utilization following myocardial infarction. J. Clin. Invest. 54: 501, 1974.

4. Lichtman, M.A., Cohen, J. and Young, J.A. Reduced arterial oxygen flow and respiratory alkalosis following myocardial infarction: Role of hemoglobin-oxygen affinity in the maintenance of oxygen consumption. Trans. Assoc. Amer. Phys. 87: 233, 1974.

5. Lichtman, M.A. The effect of hemodialysis on intraerythrocytic phosphate compounds and oxygen binding to hemoglobin. Kidney International Suppl. 2: S-134, 1975.

6. Lichtman, M.A., Whitbeck, A.A. and Murphy, M.S. Factitious changes in binding of oxygen to hemoglobin when based on extracellular pH in the presence of certain blood additives like radiographic contrast media. Invest. Radiol. 10: 225, 1975.

7. Lichtman, M.A., Murphy, M.S. and Pogal, M. The use of a single venous blood sample to assess oxygen binding to hemoglobin. Brit. J. Haemat. In press.

8. Lichtman, M.A., Murphy, M.S., Whitbeck, A.A., Pogal, M., and Lipchick, E.O. Acidification of plasma by the red cell due to radiographic contrast materials. Circulation. In press.

9. Lichtman, M.A., and Murphy, M.S. Red cell adenosine triphosphate in hypoproliferative anemia with and without chronic renal disease: Relationship to hemoglobin deficit and plasma inorganic phosphate (Proceedings of a conference on Stress Erythropoiesis, May, 1975, Rochester, New York) Blood Cells. In press.

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